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Changes in levels of biomarkers of exposure observed in a controlled study of smokers switched from conventional to reduced toxicant prototype cigarettes

Christopher J. Shepperd [a,*], Alison Eldridge [a], Oscar M. Camacho [a], Kevin McAdam [a], Christopher J. Proctor [a], Ingo Meyer [b]

[a] Group Research and Development, British American Tobacco (Investments) Ltd., Southampton, United Kingdom
[b] Momentum Pharma Services, Hamburg, Germany

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ABSTRACT

Reduced toxicant prototype (RTP) cigarettes with substantially reduced levels of tobacco smoke toxicants have been developed. Evaluation of these prototype cigarettes included measurement of biomarkers of exposure (BoE) to toxicants in smokers switched from conventional cigarettes to the RTPs. A 6-week single-blinded randomised controlled study with occasional clinical confinement was conducted (Trial registration: ISRCTN7215735). All smoking subjects smoked a conventional cigarette for 2-weeks. Control groups continued to smoke the conventional cigarette while test groups switched to one of three RTP designs. Clinical confinement and additional assessments were performed for all smoking groups after 2 and 4-weeks. A non-smoker group provided background levels of BoE. On average, smokers switched to RTPs with reduced machine yields of toxicants had reduced levels of corresponding BoEs. For vapour phase toxicants such as acrolein and 1,3-butadiene reductions of $\geqslant$70% were observed both in smoke chemistry and BoEs. Reductions in particulate phase toxicants such as tobacco-specific nitrosamines, aromatic amines and polyaromatic hydrocarbons depended upon the technologies used, but were in some cases $\geqslant$80% although some increases in other particulate phase toxicants were observed. However, reductions in BoEs demonstrate that it is possible to produce prototype cigarettes that reduce exposure to toxicants in short-term use.

1. Background

Many epidemiological studies of tobacco smoking find that risks to health are dose related and increase particularly with duration of smoking but also with level of daily consumption (International Agency for Research on Cancer, 2007; Doll et al., 1994). Cessation generally leads to reductions in health risks, but the extent and speed of any reduction varies by disease (International Agency for Research on Cancer, 2007; Doll et al., 1994). The risks can be assumed to result from repeated and prolonged exposure to a range of tobacco smoke toxicants, which leads to a gross insult to the respiratory and cardiovascular systems (e.g. through mechanisms of inflammation or oxidative stress), or to exposure to individual or classes of toxicants that exert toxic effects through more specific mechanisms (Institute of Medicine, 2001). Whilst, there is a level of understanding of how exposure to a particular tobacco smoke constituent relates to etiology disease (Fowles and Dybing, 2003; Rodgman and Green, 2003; Cunningham et al., 2011) there remains uncertainty about which smoke constituent reductions might result in reductions in smoking-related diseases.

Reducing the health impact of tobacco use is a clear public health priority and has led to a series of regulatory and educational initiatives to persuade people not to smoke (World Health Organisation, 2011). Despite these efforts, smoking rates in the adult population in many countries remain at between 15% and 25%. Although they are declining slowly in many countries (World Health Organisation, 2011), the World Health Organisation (WHO) has forecast that there will be around 1.5 billion tobacco smokers worldwide in 2050 (World Health Organisation, 2002). Both current scientific study and public policy debate are considering whether public health gains, related to individual risks and population risks, could arise from reducing toxicant exposure in people who continue to use tobacco.

Abbreviations: BoE, biomarkers of exposure; CPD, cigarettes per day; FDA, Food and Drug Administration; MLE, mouth level exposure; RTP, reduced-toxicant prototype; TNeq, total nicotine equivalents; TSNA, tobacco-specific nitrosamines; WHO, World Health Organisation.

* Corresponding author. Address: Group Research and Development, British American Tobacco (Investments) Ltd., Regents Park Road, Southampton SO15 8TL, United Kingdom. Fax: +44 2380 588914.

E-mail addresses: jim_shepperd@bat.com (C.J. Shepperd), Alison_eldridge@bat.com (A. Eldridge), kevin_mcadam@bat.com (K. McAdam), christopher_proctor@bat.com (C.J. Proctor), ingo.meyer@mps-hamburg.com (I. Meyer).

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Reducing exposure to tobacco toxicants was a theme explored in a US Institute of Medicine report published in 2001 which sought to evaluate whether a scientific basis for tobacco harm reduction, including the development and evaluation of what was termed potential reduced-exposure products (PREPs) was feasible (Institute of Medicine, 2001). PREPs were defined as products that substantially reduce exposure to one or more tobacco smoke toxicants and as a result could be reasonably expected to reduce the risk of one or more specific diseases or other adverse health effects.

Tobacco smoke is a complex mixture of many thousands of individual substances, approximately 150 of which have been identified as toxicants (Fowles and Dybing, 2003). Various toxicological approaches have been applied to try to identify which toxicants are the most important to the various diseases caused by smoking (Fowles and Dybing, 2003; Rodgman and Green, 2003; Cunningham et al., 2011). We have for some years been developing and characterising a series of technologies that have potential to reduce some of these toxicants in tobacco and tobacco smoke (McAdam et al., 2011a; Liu and Porter, 2011; Branton et al., 2011a, b). In this study we combined some of these technologies to produce a series of reduced toxicant prototype (RTP) cigarettes that when assessed in the laboratory, using smoking machines, resulted in reductions in yields of a range of tobacco smoke toxicants, as compared to both scientific and commercial controls (McAdam et al., 2011b). The scientific controls were test products containing single technologies, whilst the commercial controls were cigarettes matched for ISO tar and nicotine yield, available on the German market at the time of the study.

It is well known that the yield of toxicants produced when a cigarette is smoked using a smoking machine will depend on the way in which the machine is set up to smoke the cigarette, and is particularly dependent on the size and the frequency of the puffs. Larger and more frequent puffs mean more tobacco is burnt in the creation of the mainstream smoke and hence the levels of the toxicants produced are increased. This is important because it is also well known that human smoking behaviour (including the number and size of puffs taken) varies widely, both from occasion to occasion for a particular smoker and between smokers. Therefore, laboratory machine yields will not necessarily reflect toxicant exposure in an individual smoker or a population of smokers. Even so, several studies have correlated levels of biomarkers of exposure (BoE), such as toxicant metabolites, with levels of toxicants in the smoke collected on smoking machines. For example, Ashley reported lower levels of BoE associated with tobacco specific nitrosamines (TSNA) in groups of smokers using cigarettes with low TSNA tobacco blends (Ashley et al., 2010). In another study, Sarkar reported reductions in BoE associated with several volatile tobacco smoke toxicants in subjects switched from conventional cigarettes to cigarettes with activated carbon (an adsorbent of volatile compounds) added to the filter (Sarkar et al., 2008).

Three RTPs (Table 1), each with different relative levels of toxicant yields, have been used in this study to test the hypothesis that the reduced yields of some toxicants in mainstream smoke would lead to reduced levels of corresponding BoE in smokers, thereby indicating a reduction in smoke toxicant exposure.

The smoke toxicants followed in this study were determined by the availability of established BoE methods, therefore it was not possible to follow all of the smoke toxicant reductions associated with these RTPs (McAdam et al., 2011b). The BoE and associated smoke toxicants assessed in this study can be found in Table 4.

The primary objective of the study was to estimate and compare cigarette smoke exposure in healthy adult smokers by assessment of BoE and mouth level exposure (MLE) to tar and nicotine as measured by filter analysis, and to quantify any changes in exposure after switching from control cigarettes to RTPs.

Secondary objectives of the study were to determine changes in a variety of measures during consumption of RTP cigarettes and reference cigarettes using sensory testing questionnaire, assessments of smoking behaviour, including puffing and inhalation behaviour, a quality of life (QoL) questionnaire, and basic physiological measures.

This paper focuses on the primary objective of the study.

2. Materials and methods

A six week, single-centre, single-blinded, controlled, forced switch clinical study was conducted at Momentum Pharma Services, Hamburg, Germany. The study was designed and conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Committee on Harmonisation for Good Clinical Practice. The protocol was approved by the ethics committee of the Artzekammer Hamburg and the study conducted between December 2008 and June 2009. All subjects provided written informed consent. The study is registered with Current Controlled Trials, number ISRCTN72157355.

2.1. Subjects

Subjects were recruited into the study using a combination of the clinic's own website and local advertising that did not refer to the characteristics of the study products. Eligible subjects were healthy adult (>21 years) smokers and non-smokers of either sex and any ethnic origin who lived in or around Hamburg, Germany. Eligibility was assessed by the principal investigator on the basis of the following criteria.

Inclusion criteria for smokers required subjects: to be regular smokers of either 6–7 mg or 1–2 mg ISO tar yield cigarettes; to have typically self-reported cigarette consumption of between 6 and 30 cigarettes per day; to have been a smoker for >3 years; to have smoked their current brand for >6 months (where the current brand was typical of the German market in terms of format ("king size"), blend style (American blended, non-mentholated) and filter type (plain cellulose acetate)); and to be willing to switch to a novel product. Eligible non-smokers were required to have not smoked for >5 years and have a urine cotinine level of <10 ng/mL.

Universal exclusion criteria were as follows: a clinically relevant health condition or abnormal findings on physical examination; participation in a clinical trial within 90 days of day 1; donation or loss of $\geqslant$400 mL blood in the past 90 days; acute illness requiring treatment within the previous 4 weeks; use of nicotine or tobacco products other than filtered cigarettes; any history of drug or alcohol abuse; use of bronchodilators within the previous 12 months; use of systemic medication (except hormonal contraception or hormone-replacement therapy) within the previous 14 days; employment in the tobacco, journalism, public relations, market research or advertising industries; a positive urine pregnancy test, use of non-reliable contraceptive methods or lactation in women of childbearing age. Smokers were excluded if they self-reported or were observed to be non-inhalers.

All subjects were screened for study entry by physical examination, electrocardiography, clinical laboratory tests, lung function tests, medical history, urinary cotinine test (non-smokers), the ability to understand the study protocol and give written informed consent to participate and a willingness to refrain from consuming products containing caffeine for 24 h and alcohol for 72 h before the first day of each confinement visit.

2.2. Products

Five products were manufactured for this study (Table 1). Further details of the designs and associated smoke chemistries of all five products have been published previously (McAdam et al.,

Table 1
Construction characteristics and smoke yields* for control and reduced-toxicant prototype cigarettes used in the study.

	CC1	TSS1	BT1	CC6	TSS6
Tobacco blend composition	US style	80% US style/20% TSS	75.4% washed, extracted and enzyme treated tobacco/20.3% Virginia tobacco/4.3% oriental tobacco	US style	80% US style/20% TSS
Cigarette rod length (mm)	57	57	57	57	57
Blend weight (mg)	570	572	654	605	622
Cigarette paper	50 CU standard	50 CU standard	50 CU standard	50 CU standard	50 CU standard
27 mm filter type	Single-stage	Three-stage	Three-stage	Single-stage	Two-stage
Ventilation (%)	78	81	79	52	46
Pressure drop (mmWG)	86	97	91	85	109
Mouth-end stage	27 mm CA	7 mm CA	7 mm CA	27 mm CA	15 mm CA
Middle stage		10 mm CA with 20 mg CR20L	10 mm CA with 20 mg CR20L		
Tobacco end stage		10 mm CA with 60 mg C	10 mm CA with 60 mg C		12 mm CA with 80 mg C
Target ISO Tar yield (mg)	1	1	1	6	6
NFDPM (mg/cig)	18.9	17.3	17.8	24.4	20.7
Nicotine (mg/cig)	1.3	1.2	1.5	1.6	1.4
Nitrosamines (ng/cig)					
NAB (RL = 0.1 ng/cig)	13.6	6.6	1.4	12.1	7.6
NAT (RL = 0.2 ng/cig)	124.5	70.3	19.1	117.6	69.5
NNK (RL = 0.2 ng/cig)	57.9	48.2	10.1	80.0	44.5
NNN (RL = 0.2 ng/cig)	245.2	76.0	10.2	146.9	72.8
Aromatic amines (ng/cig)					
2-Aminonaphthalene (RL = 0.5 ng/cig)	13.1	11.5	7.4	14.6	14.8
3-Aminobiphenyl (RL = 0.1 ng/cig)	3.5	3.0	1.8	4.1	3.3
4-Aminobiphenyl (RL = 0.1 ng/cig)	2.8	2.5	1.2	3.1	2.7
o-Toluidine (RL = 2.3 ng/cig)	68.1	60.1	50.6	88.1	76.2
Carbonyls (μg/cig)					
Acrolein (RL = 1.0 μg/cig)	130.5	52.5	75.0	139.4	62.3
Crotonaldehyde (RL = 1.1 μg/cig)	41.6	6.0	10.9	45.2	7.0
Hydrocarbons and PAH (μg/cig)					
1,3-Butadiene (RL = 7.0 μg/cig)	39.6	27.2	53.4	63.6	36.8
Naphthalene (RL = 62.5 μg/cig)	2182.5	643.8	484.9	2952.3	565.6
Fluorene (RL = 62.5 μg/cig)	230.5	148.3	247.3	315.7	240.9
Phenanthrene (RL = 62.5 μg/cig)	524.4	191.4	541.5	739.8	589.7
Pyrene (RL = 9.4 μg/cig)	70.4	64.6	75.3	108.1	80.3

Abbreviations: TSS = tobacco substitute sheet; BT = blend treatment; CA = cellulose acetate filter material; CR20L = amine-functionalised resin filter additive; C = high-activity polymer-derived carbon; CU = CORESTA unit (volume air [cm^3] passing through 1 cm^2 paper min^{-1} at constant pressure difference of 1.0 kPa). NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine, NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; 3-HPMA = 3-hydroxypropylmercapturic acid; PAH = polynuclear aromatic hydrocarbons. RL = reporting limit.
* Measured under the Health Canada Intense smoking regime [10].

2011b) and toxicant yields, acquired through standardised machine smoking, relevant to the current study are summarised in Table 1. Testing cigarettes under ISO smoking conditions, we found that some of the toxicant levels were below reporting limits (RL) (Table 1), and so we report in this paper smoke toxicant yields acquired after machine smoking under Health Canada Intense smoking conditions which produces higher yields. Cigarettes used in this clinical study were sourced from the same manufacturing batch as those from which the previously published data were obtained.

Two of the five products were manufactured as controls for this study, one with a 6 mg ISO tar yield (CC6) and one with a 1 mg ISO tar yield (CC1). These products were based on British American Tobacco cigarettes on sale in Germany at the time of the study and were similar to the market leading products in terms of format (''king sized''), tobacco blend style (American blended) and filter type (cellulose acetate).

The other three products were reduced toxicant prototype (RTP) cigarettes (Table 1). The RTPs used different combinations of four novel toxicant reducing technologies.

Two novel technologies were included in the tobacco blend of the prototype cigarettes with the aim of reducing levels of toxicant yields: a tobacco substitute sheet (TSS), the use of which results in a reduced amount of tobacco being burnt and the smoke being diluted by a proportion of the glycerol contained in the sheet (McAdam et al., 2011a), and a tobacco blend treatment (BT) that lowers levels of proteins and polyphenols in tobacco, resulting in reduced levels of aromatic amines, hydrogen cyanide and phenolic compounds in smoke (Liu and Porter, 2011).

In order to reduce levels of some vapour phase toxicants, two different adsorbents were used in the filters of the RTP cigarettes: a high performance carbon that had significantly better adsorption capacity than carbons typically used in the manufacture of some conventional cigarettes (Branton et al., 2011a) and an amine-functionalised resin with chemisorptive properties for carbonyls and volatile acids (Branton et al., 2011b).

Combinations of these technologies were used to develop two prototype cigarettes with target ISO tar yield of 1 mg, and one with a target ISO tar yield of 6 mg. The 1 mg ISO tar yield was chosen as it is the lowest yield category under the ISO smoking regime in Germany. The first of these 1 mg prototypes (TSS1) incorporated tobacco substitute sheet into the tobacco blend, and a three stage filter containing both the high performance carbon and amine functionalised resin in addition to cellulose acetate (the material typically used for cigarette filters). The second, (BT1) incorporated

treated tobacco into the blend and the same three stage filter as TSS1 (see Table 1).

Cigarettes of around 6 mg ISO tar yield were considerably more popular in Germany in 2007–8 than 1 mg ISO tar cigarettes, and so a further prototype was developed at this higher ISO tar yield. This prototype (TSS6) also included the tobacco substitute sheet in the blend but used a two stage filter containing only the high performance carbon in addition to the traditional cellulose acetate.

2.3. Study groups

Subjects who smoked 6–7 mg ISO tar yield cigarettes were assigned to the 6 mg control group, which smoked CC6 throughout the study (group 1), or to the 6 mg RTP group, which switched from CC6 to TSS6 (group 2). The subjects who smoked 1–2 mg ISO tar yield cigarettes were assigned either to the 1 mg control group, which smoked CC1 throughout the study (group 3), or to one of two 1 mg RTP groups, which switched from CC1 to either the TSS1 RTP (group 4) or the BT1 RTP (group 5). Group 6 comprised the non-smoking control group (Fig. 1). Group assignment was not based on formal randomisation, but participants were assigned to subgroups within the ISO tar yields (i.e. control or test group) to ensure age and sex were matched as closely as possible (see Table 2). Smoking participants were unaware of group allocation throughout the study.

2.4. Study protocol

The study was mainly ambulatory, but included short periods of clinical confinement when samples were collected for analysis (Fig. 1). On days 1, 7, 21 and 35, smokers made ambulatory visits to the clinic to collect supplies of cigarettes. For each ambulatory period, individuals were supplied with a number of cigarettes equal to their self-reported daily consumption plus two further packs; when consumption values were recorded, subjects did not know that supplies would be based on these values. All cigarettes were supplied unbranded and in plain white packs bearing health warnings and a four-digit alphanumerical code referencing the product, which also appeared on each cigarette. To assess compliance during ambulatory periods, subjects completed daily diaries of cigarette consumption. They were also required to collect the butts of smoked cigarettes each day and return them along with any unsmoked cigarettes. Although subjects were asked to smoke only supplied cigarettes for the duration of the study, an "honesty" policy was used to enable them to record any other cigarettes smoked without risk of expulsion from the study. During these ambulatory periods, no restrictions were placed on when and how many cigarettes were smoked and no samples were taken for analysis. On day 1, subjects were advised to refrain from grilled, smoked, fried or barbecued food and cruciferous vegetables during non-residential parts of the study, and were instructed to document the consumption of such foods in a diary.

On days 1–14, all smokers received CC6 or CC1 control cigarettes according to group allocation. On the evening of day 12, smokers entered the clinic for the first period of clinical confinement, during which cigarettes were issued singly on request after the filter of the previous smoked cigarette had been returned. This approach ensured complete collection of filters and accurate cigarette consumption data. On the evening of day 14, group 2 was switched from CC6 to TSS6, group 4 from CC1 to TSS1 and group 5 from CC1 to BT1. Groups 1 and 3 continued to smoke CC6 and CC1 respectively. All smokers continued to smoke these supplied cigarettes for the remaining 4 weeks of the study.

On the evening of day 15 all smoking subjects left the clinic with sufficient cigarettes to last until their next ambulatory visit on day 21. Further clinical confinement periods took place for these groups on days 26–28 and 38–42.

Non-smokers completed two periods of clinical confinement that bracketed, in time, the smoking groups (days 5–6 and 54–56 of the study). At no point were non-smokers in clinic at the same time as smokers and indeed each smoking group (1–5) occupied the clinic at different times, ensuring no mixed groups of control and test, or 6 mg and 1 mg ISO tar yield.

For all subjects, to minimise potential dietary interferences on BoE levels, restrictions were placed on caffeine and alcohol intake for 24 h and 72 h respectively before the start and to the end of the confinement periods, and subjects received a standardised bulk diet (excluding grilled, smoked, fried and barbequed foods) during stays at the clinic.

During all confinement visits, 24 h urine samples were taken for BoE analysis from all subjects. Although clinical confinement virtually ensured complete 24 h urine sample collection, completeness was confirmed using consistency of creatinine clearance values, calculated from serum and urine creatinine measures taken at each 24hr period. Twenty-one urinary BoE were measured for tobacco smoke toxicants for which validated methods were available (Tables 4 and 5). The methods utilised for BoE for tobacco-specific nitrosamines, aromatic amines, crotonaldehyde and polyaromatic hydrocarbons (PAHs) have been reported elsewhere (Kavvadias et al., 2009; Riedel et al., 2006; Scherer et al., 2007; Ramsauer et al., 2011) and the methods utilised for BoE of nicotine and metabolites, acrolein and 1,3-butadiene were adapted from published methods (Sarkar et al., 2008; Feng et al., 2007; Sheldon, 2003). Levels were reported as the quantity of BoE excreted over 24 h, and were calculated as the product of urine BoE concentration and the volume of urine. Saliva samples were collected and analysed as previously described (Shepperd et al., 2009), to measure salivary cotinine as a check on compliance with the protocol. Urinary BoE results for day 14 for smokers were treated as baseline values, results for days 28 and 41 were treated as mid-point and

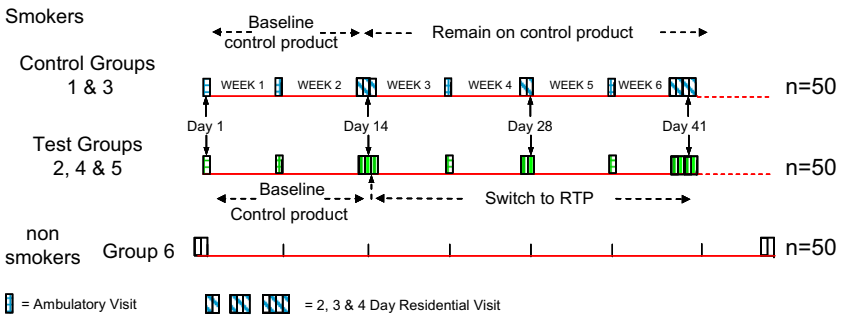

Fig. 1. Study design. Key: Blue shading – Control Groups, Green shading – Test Groups, No Shading – non-smokers.

Table 2
Characteristics of participants by study group.

	Group 1 (CC6; $n = 50$)	Group 2 (TSS6; $n = 50$)	Group 3 (CC1; $n = 51$)	Group 4 (TSS1; $n = 50$)	Group 5 (BT1; $n = 50$)	Group 6 (non-smokers; $n = 50$)
Enrolled (n)	50	50	51	50	50	50
Completed (n)	49	49	45	47	47	48
Per protocol population (n)	46	49	42	45	47	48
Gender						
Male	23 (50%)	23 (47%)	20 (48%)	24 (53%)	23 (49%)	24 (50%)
Female	23 (50%)	26 (53%)	22 (52%)	21 (47%)	24 (51%)	24 (50%)
Age (years)						
21–30	13 (28%)	19 (39%)	13 (31%)	9 (20%)	15 (32%)	9 (19%)
31–60	30 (65%)	27 (55%)	25 (60%)	31 (69%)	28 (60%)	26 (54%)
>60	3 (7%)	3 (6%)	4 (9%)	5 (11%)	4 (8%)	13 (27%)
Mean ± SD	38 ± 10	38 ± 13	39 ± 13	46 ± 12	43 ± 12	47 ± 16
Ethnicity						
Caucasian	44	48	42	45	47	47
Non-caucasian	2	1	0	0	0	1

end-point values for smokers and results for day 56 for non-smokers were taken as non-smoking control values.

For smoking subjects, cigarette filters were collected for the 24 h periods that corresponded with urine collection and were used to assess nicotine mouth level exposure (MLE, an estimate of the amount of nicotine that exits the cigarette filter and enters the mouth) with a previously described part-filter analysis procedure (St Charles et al., 2006). On days 14, 15, 28 and 42, smokers also completed a questionnaire, adapted for this study, on 11 sensory characteristics of the product, including draw resistance, irritation and acceptability using subjective questions scored on a five point scale (McAdam et al., 2011a).

2.5. Statistical analysis

Sample size estimates were based on observed percentage reductions in smoke toxicants from CC1 to BT1 and TSS1 (given that the 1 mg RTPs gave the smallest absolute reductions in smoke toxicants from the control cigarette). Assuming that these reductions would translate into corresponding BoE reductions, historical biomarker data were used to estimate the effect size based on the smallest anticipated change (aromatic amines). Sample size calculations were carried out using the sample size calculator for a two sample parity T-test in MINITAB v15 software. We determined that to achieve power of 80% in the study, a sample size of 50 would be adequate for all BoE of interest.

Assessments of changes in BoE levels compared values at baseline with values at mid-point and end of study. BoE data at mid-point and end of study were compared for each analyte and group to assess whether any changes noted after two weeks were maintained throughout the study.

In all cases, if normality or homogeneity of the variance assumptions were not respected, a log-transformation or non-parametric approach was applied. Repeated measures analyses of variance (ANOVA) or covariance (ANCOVA) were done with SAS PROC MIXED, on the change from baseline for each BoE, with day-∗group interaction as a fixed effect. The repeated measures were taken into account, with the term subject nested within group as a random effect, with use of the SAS repeated statement. Significance of the interaction was evaluated at $\alpha = 0.05$. If the day∗group interaction was significant, the term was maintained in the model and the group comparison was performed separately for each day. Otherwise the interaction was removed and the group comparison was assessed globally. ANCOVA was considered if absolute exposure measurement values could be used. The same model as for ANOVA was used, but the number of smoked cigarettes was applied as a continuous covariate. ANCOVA was used if the number of cigarettes per day (CPD) changed over time and a significant relationship was seen between BoE values and CPD that was not dependent on the fixed effects (day and group). Details of the statistical analysis plan for this study are available from the authors on request.

All statistical analyses were performed with SAS DD© (UNIX) Version 9.1 or higher, and edit checks were performed on OpenVMS platform SAS Version 8.2.

3. Results

Three hundred and one subjects were recruited, of whom 100 smoked 6–7 mg ISO tar yield cigarettes, 151 smoked 1–2 mg ISO tar yield cigarettes, and 50 were non-smokers. Characteristics of subjects are shown in Table 2. Of these, 285 (95%) completed the study. Sixteen did not complete the study because of personal reasons ($n = 8$), protocol violations ($n = 4$), pregnancy ($n = 2$), non-compliance to the study protocol ($n = 1$), and an adverse event (not related to study) ($n = 1$). A further eight subjects were removed from the analysis because of an incomplete data set for those subjects. Therefore, the per-protocol population comprised 277 subjects (Table 2).

3.1. Demographic data

The distribution of subjects with respect to age and gender in the per protocol set was similar across and within study groups (Table 2). The mean ages for the two 6 mg groups were identical (38 years). There was some variation between the mean ages for the three 1 mg groups (39–46 years). However, standard deviation was similar for all groups. Absolute matching of age was not essential since 6 mg and 1 mg groups were not compared, and with the tar categories the primary comparisons are within group rather than between groups.

3.2. Cigarette consumption and nicotine MLE

Self-reported cigarette consumption was recorded at screening to meet the inclusion/exclusion criteria, but no measures of consumption or intake were made on subjects own brands prior to day 1. After day 1, actual cigarettes per day (CPD) were recorded at multiple time-points during the study and are shown graphically in Fig. 2. Day to day cigarette consumption was variable throughout the study for all groups, with no obvious trends, with the exception of a noticeable uplift in consumption for all groups

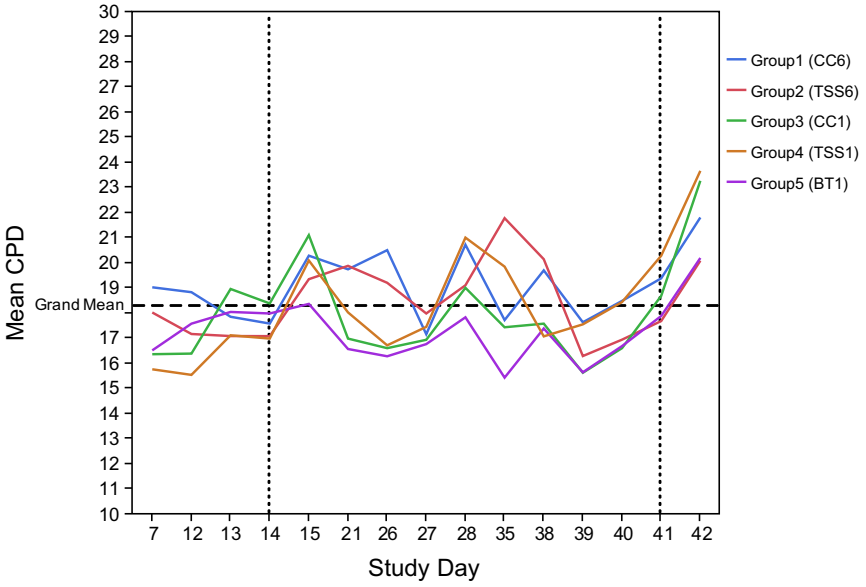

Fig. 2. Mean cigarette consumption through Study for all groups.

Table 3
Cigarette consumption and nicotine mouth level exposure at baseline and study end, by study group.

Group	LS Mean consumption cigarettes per day				LS Mean MLE nicotine (mg/24 h)			
	Day 14	Day 41	Percentage change (%)	*p* value	Day 14	Day 41	Percentage change (%)	*p* value
1 (CC6)	17.6 ± 5.6	19.4 ± 7.1	+10	0.0001	20.3 ± 8.5	23.5 ± 11.2	+16	0.0008
2 (TSS6)	17.1 ± 6.7	17.7 ± 7.7	+3	0.1697	21.4 ± 11.8	23.5 ± 14.1	+10	0.0241
3 (CC1)	18.4 ± 5.1	18.7 ± 5.8	+2	0.5687	12.0 ± 7.2	14.5 ± 8.3	+21	0.0014
4 (TSS1)	17.0 ± 6.5	20.3 ± 7.3	+19	<0.0001	10.6 ± 6.8	18.5 ± 10.4	+74	<0.0001
5 (BT1)	18.0 ± 5.8	17.9 ± 7.9	−0.5	0.9172	9.8 ± 6.0	17.0 ± 10.9	+73	<0.0001

in the last days of the study (including day 41), which was only statistically significant for groups 1 and 4 (Table 3). This end-of-study uplift has been noted in previous studies (Shepperd et al., 2009; St Charles et al., 2006) and could be attributable to the imminent study end and hence the supply of test cigarettes. Because of this, day 41 was selected for analysis as the end of study timepoint rather than day 42. With the exception of this end-of-study effect, statistical analysis of cigarette consumption suggests that consumption remained reasonably stable throughout the study, and that subjects were acclimatised to control cigarettes within the initial 14 day period (Fig. 2).

CPD and nicotine MLE data for all smoking groups for baseline (day 14) and end of study (day 41) are shown in Table 3. At baseline, CPD for all groups was 17–18.4, with no significant difference between the groups.

The mean values for MLE nicotine at baseline were similar for groups 1 and 2 at around 20 mg/24 h and for groups 3, 4 and 5, at around 11 mg/24 h reflecting differences in the nicotine yield of the two control products. Nicotine MLE increased in all groups from baseline to end of study (Table 3). The magnitude of this increase was consistent with the changes seen in total nicotine equivalents (TNeq) for the 6 mg groups (Table 4), but was two to three times higher than those seen for the 1 mg groups (Table 5).

3.3. Biomarkers of exposure

Tables 4–6 detail the biomarker of exposure data for 6 mg groups, 1 mg groups and non-smokers, respectively. Results for selected BoE are also presented as boxplots in Fig. 3. Tables 7–9 detail the smoke yield and BoE data expressed as absolute values and% change between test and control (smoke data) or between baseline and end of study (BoE) Table 6.

3.3.1. 6 mg Groups

In group 1 (CC6) end-of-study levels were higher than at baseline for all BoE, except those for 3-hydroxyphenanthrene and 4-hydroxyphenanthrene, which decreased, and for DHBMA, which did not change (Table 4). Increases were significant except those for NNN, 3-aminobiphenyl, 2-hydroxyphenanthrene and 3-hydroxyphenanthrene.

By contrast, subjects in group 2 (TSS6) showed reductions in all biomarkers at end of study compared with baseline except for TNeq and 4-hydroxyphenanthrene. All reductions were significant except those for 4-aminobiphenyl, 1-hydroxypyrene and 2-hydroxyphenanthrene, 3-hydroxyphenanthrene and 4-hydroxyphenanthrene. TNeq was significantly higher at end of study compared to baseline, despite no significant increase in CPD. The greatest reductions were seen for the volatile compounds, which were reduced by 75%, 45% and 63% for crotonaldehyde, acrolein and 1,3-butadiene (MHBMA) BoEs respectively. BoEs for the TSNAs were reduced by between 10% and 26%.

3.3.2. 1 mg Groups

In group 3 (CC1), most BoE levels were similar at baseline and day 41. Statistically significant increases were observed for some BoEs, including acrolein (3-HPMA) and 2-aminonapthalene (Table 5).

Table 4
LS Means, SD, ranges and comparisons (p-values) for tobacco biomarkers of exposure in 6 mg cigarette groups.

Smoke constituent	Biomarker*	Group 1 (CC6)		% Change 14 to 41	Group 2 (TSS6)		% Change 14 to 41	Group 2 vs Group 1 p-values	
		Day 14	Day 41		Day 14	Day 41		Day 14	Day 41
Nicotine	TNeq (mg/24 h)	12.0 ± 5.9 (0.2–23.6) p = 0.0047	13.6 ± 6.6 (0.4–29.0)	+13	11.7 ± 7.0 (2.1–30.6) p=0.0253	12.9 ± 8.9 (1.4–38.6)	+10	0.8344	0.6429
NNK	NNAL (ng/24 h)	287 ± 164 (13–678) p = <0.0001	377 ± 196 (2–981)	+31	315 ± 184 (64–652) p = 0.0200	282 ± 175 (37–667)	−10	0.442	0.0098
NNN	NNN (ng/24 h)	13.9 ± 11.4 (0.4–55.7) p = 0.6108	14.6 ± 10.7 (0.4–63.8)	+5	14.8 ± 8.8 (0.4–33.4) p = 0.0174	11.5 ± 7.6 (0.3–46.0)	−22	0.8044	0.3714
NAB	NAB (ng/24 h)	29.8 ± 19.8 (0.6–84.2) p = <0.0001	39.0 ± 24.7 (0.6–110)	+31	35.3 ± 27.5 (0.6-105.5) p = <0.0001	26.2 ± 20.4 (0.5–99)	−26	0.2205	0.0050
NAT	NAT (ng/24 h)	160 ± 116 (2.4–448) p = <0.0001	214 ± 137 (2.6–611)	+34	187 ± 144 (0.4–527) p = <0.0001	143 ± 109 (0.3–495)	−24	0.2813	0.0044
4-Amino biphenyl	4-Aminobiphenyl (ng/24 h)	13.9 ± 7.1 (3.1-33.0) p = 0.0202	15.4 ± 8.1 (3.6-48.8)	+11	15.7 ± 8.8 (3.6–39.6) p = 0.1287	14.7 ± 9.0 (1.9–45.0)	−6	0.2810	0.6681
3-Amino biphenyl	3-Aminobiphenyl (ng/24 h)	6.6 ± 3.7 (03–15.5) p = 0.2511	6.9 ± 4.9 (0.8–27.3)	+5	6.8 ± 4.1 (1.0–16.6) p = 0.0086	6.1 ± 3.5 (0.7–14.6)	−10	0.7408	0.1997
o-Toluidine	*o*-Toluidine (ng/24 h)	149 ± 62.3 (40–277) p = 0.0204	163 ± 64.2 (43.0–315.4)	+9	143 ± 61.6 (52–291) p = 0.0371	131 ± 56.5 (48.3–244.1)	−8	0.6352	0.0138
Crotonaldehyde	HMPMA (μg/24 h)	1046 ± 532 (217–2293) p = <0.0001	1429 ± 636 (362–3186)	+37	1215 ± 725 (226–3867) p = <0.0001	308 ± 150 (101–698)	−75	0.1276	<0.0001
2-Amino naphthalene	2-Amino naphthalene (ng/24 h)	21.6 ± 10.9 (1.3–45.1) p = <0.0001	26.1 ± 13.9 (1.5–82.6)	+21	23.7 ± 14.9 (5.1–72.3) p = 0.0136	21.1 ± 14.3 (3.0–56.1)	−11	0.4526	0.0705
Fluorene	2-Hydroxy fluorene (ng/24 h)	2562 ± 1179 (529–4959) p = 0.0057	2852 ± 1333 (723-6952)	+11	2587 ± 1395 (665–6943) p = 0.2130	2232 ± 1350 (449–6206)	−14	0.9269	0.0205
Pyrene	1-Hydroxy pyrene (ng/24 h)	254 ± 101 (71–511) p = 0.011	287 ± 125 (110-756)	+13	283 ± 157 (57–634) p = 0.0005	267 ± 19 (61–881)	−6	0.3134	0.4860
Naphthalene	1-Hydroxy naphthalene (ng/24 h)	6569 ± 3156 (375–13332) p = 0.0004	7772 ± 3645 (434-17951)	+18	7103 ± 4526 (1379–20983) p = 0.0015	6065 ± 4134 (817–20318)	−15	0.5019	0.0327
Naphthalene	2-Hydroxy naphthalene (ng/24 h)	13540 ± 6417 (2471–35849) p = 0.0009	15695 ± 6423 (3536-33809)	+16	13904 ± 7607 (3969–35525) p = 0.0111	12323 ± 7421 (2577–33485)	−11	0.7960	0.0173
Acrolein	3-HPMA (ng/24 h)	1342 ± 743 (18.9–2903.6) p = 0.0001	1803 ± 882 (338-4241)	+34	1365 ± 874 (272–3782) p = 0.0001	751 ± 486 (226–2388)	−45	0.884	<0.0001
1,3-Butadiene	MHBMA (ng/24 h)	4501 ± 3614 (376–18739) p = 0.0031	5385 ± 4568 (302-23820)	+20	4028 ± 3961 (94–17490) p = 0.0001	1487 ± 1729 (78–8456)	−63	0.5112	<0.0001
1,3-Butadiene	DHBMA (μg/24 h)	484 ± 156 (205–846) p = 0.9852	484 ± 138 (240-832)	0	440 ± 158 (150–811) p = 0.0305	407 ± 132 (193–736)	−8	0.1374	0.0091
Phenanthrene	2-Hydroxy phenanthrene (ng/24 h)	119 ± 47 (30–224) p = 0.1484	124 ± 50 (43-255)	+4	123 ± 46 (38–267) p = 0.2463	108 ± 47 (31–263)	−4	0.5425	0.0558
Phenanthrene	3-Hydroxy phenanthrene (ng/24 h)	240 ± 92 (79–441) p = 0.1094	226 ± 83 (86-438)	−6	217 ± 87 (72–459) p = 0.2263	207 ± 93 (44–457)	−5	0.1211	0.2016
Phenanthrene	4-Hydroxy phenanthrene (ng/24 h)	183 ± 155 (27–684) p = 0.0001	74 ± 30 (17-174)	−60	55 ± 26 (17–123) p = 0.2894	68 ± 31 (15–157)	−24	<0.0001	0.7025
Phenanthrene	1+9-Hydroxy phenanthrene (ng/24 h)	570 ± 226 (166–1025) p = 0.0373	625 ± 259 (235-1269)	+10	578 ± 253 (203–1256) p = 0.0426	526 ± 251 (178–1411)	−9	0.881	0.0489

* TNeq = total nicotine equivalents. NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine, NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; HMPMA = 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA = 3-hydroxypropylmercapturic acid; DHBMA = 1,2-dihydroxybutyl mercapturic acid; MHBMA = monohydroxybutenyl-mercapturic acids.

Table 5
LS Means, SD, ranges and comparisons (*p*-values) for tobacco biomarkers of exposure in 1 mg cigarette groups.

Smoke constituent	Biomarker*	Group 3 (CC1)		% Change 14–41	Group 4 (TSS1)		% Change 14 to 41	Group 5 (BT1)		% Change 14 to 41	Group 4 vs 3 *p*-values		Group 5 vs 3 *p*-values	
		Day 14	Day 41		Day 14	Day 41		Day 14	Day 41		Day 14	Day 41	Day 14	Day 41
Nicotine	TNeq (mg/24 h)	8.9 ± 4.4 (1.9–22.1) *p* = 0.0706	9.6 ± 4.7 (1.6–19.8)	+8	7.3 ± 4.6 (0.7–20.5) *p* = <0.0001	9.8 ± 5.2 (0.6–20.8)	+34	8.0 ± 4.5 (1.0–17.9) *p* = <0.0001	9.8 ± 6.2 (1.0–26.1)	+23	0.1622	0.9117	0.4448	0.8636
NNK	NNAL (ng/24 h)	280 ± 130 (91–608) *p* = 0.0867	305 ± 156 (89–658)	+9	207 ± 131 (18–535) *p* = <0.0001	271 ± 168 (64–751)	+31	238 ± 131 (45–673) *p* = <0.0001	128 ± 65 (2–284)	−46	0.012	0.2353	0.142	<0.0001
NNN	NNN (ng/24 h)	15.1 ± 10.8 (0.9–52.6) *p* = 0.1040	17.6 ± 13.4 (1.3–65.4)	+17	12.2 ± 9.0 (0.4–43.9) *p* = 0.0101	8.3 ± 7.4 (0.6–37.3)	−32	15.8 ± 17.7 (1.3–116) *p* = <0.0001	2.2 ± 3.2 (0.4–20.2)	−86	0.2193	<0.0001	0.7324	<0.0001
NAB	NAB (ng/24 h)	29.4 ± 18.7 (3.4–103) *p* = 0.0011	35.6 ± 22.9 (4.7–103)	+21	23.5 ± 16.2 (0.5–56.9) *p* = 0.1051	20.5 ± 14.4 (1.1–60.1)	−13	30.1 ± 21.1 (1.8–97.4) *p* = <0.0001	5.9 ± 4.2 (1.0–18.4)	−80	0.1025	<0.0001	0.8514	<0.0001
NAT	NAT (ng/24 h)	180 ± 118 (14–575) *p* = 0.0006	224 ± 150 (26–600)	+24	144 ± 112 (1–491) *p* = 0.0004	99 ± 74 (1–305)	−31	186 ± 139 (1–645) *p* =< 0.0001	28.5 ± 23.1 (1–104)	−85	0.1252	<0.0001	0.8061	<0.0001
4-Amino biphenyl	4-Amino biphenyl (ng/24 h)	12.2 ± 5.3 (3.3–21.1) *p* = 0.0891	13.1 ± 5.4 (3.3–24.3)	+7	10.1 ± 4.7 (2.2–23.2) *p* = <0.0001	12.6 ± 6.2 (2.1–26.9)	+25	11.1 ± 5.9 (2.4–24.8) *p* = <0.0001	6.9 ± 3.4 (1.7–16.1)	−38	0.0701	0.6585	0.3135	<0.0001
3-Amino biphenyl	3-Amino biphenyl (ng/24 h)	5.1 ± 3.5 (0.6–13.8) *p* = 0.0230	5.7 ± 3.6 (0.7–14.2)	+12	4.2 ± 2.8 (0.2–12.3) *p* = <0.0001	5.4 ± 3.5 (0.5–15.5)	+29	4.3 ± 2.2 (0.6–10.5) *p* = <0.0001	2.7 ± 1.4 (0.5–6.3)	−37	0.1665	0.6388	0.2282	<0.0001
o-Toluidine	*o*-Toluidine (ng/24 h)	133 ± 47 (66–273) *p* = 0.0152	146 ± 55 (62–282)	+10	122 ± 44 (34–242) *p* = 0.0471	111 ± 43 (44–224)	−9	130 ± 50 (55–282) *p* = 0.2749	124 ± 49 (51–258)	−5	0.1961	<0.0001	0.6927	0.0107
Crotonaldehyde	HMPMA (mg/24 h)	889 ± 521 (216–2558) *p* = 0.0845	980 ± 528 (235–2179)	+10	680 ± 450 (164–2356) *p* = <0.0001	288 ± 135 (115–769)	−58	741 ± 401 (153–1657) *p* = <0.0001	275 ± 108 (108–545)	−62	0.016	<0.0001	0.0828	<0.0001
2-Amino naphthalene	2-Amino naphthalene (ng/24 h)	17.5 ± 7.6 (4.1–34.8) *p* = <0.0001	21.0 ± 10.1 (5.5–41.9)	+20	15.0 ± 8.2 (1.7–36.0) *p* = <0.0001	19.6 ± 10.6 (2.4–44.8)	+30	16.3 ± 9.3 (0.8–38.7) *p* = 0.0011	13.8 ± 7.7 (2.3–34.7)	−15	0.2012	0.5057	0.5462	0.0003
Fluorene	2-Hydroxy fluorene (ng/24 h)	1968 ± 818 (390–3838) *p* = 0.0434	2132 ± 986 (583–4379)	+8	1627 ± 888 (327–4197) *p* = 0.1151	1751 ± 924 (223–3883)	+8	1794 ± 891 (465–4639) *p* = <0.0001	2134 ± 1136 (443–4773)	+19	0.1025	0.0695	0.3991	0.9908
Pyrene	1-Hydroxy pyrene (ng/24 h)	246 ± 160 (49–829) *p* = 0.8589	244 ± 179 (55–1122)	−<1	175 ± 77 (33–372) *p* = 0.2833	190 ± 83 (63–422)	+9	190 ± 95 (51–545) *p* = 0.5871	197 ± 109 (40–575)	+4	0.0031	0.026	0.0168	0.0473
Naphthalene	1-Hydroxy naphthalene (ng/24 h)	5090 ± 2657 (1097–11,986) *p* = 0.2180	5443 ± 2302 (1189–10,005)	+7	4201 ± 2672 (267–12,370) *p* = 0.9654	4213 ± 3069 (305–15,623)	+<1	4847 ± 2955 (354–12,522) *p* = 0.0010	5744 ± 3558 (902–16,202)	+16	0.0703	0.0126	0.6152	0.5333
Naphthalene	2-Hydroxy naphthalene (ng/24 h)	9994 ± 5002 (2657–26,308) *p* = 0.0631	11,067 ± 6246 (3186–36,739)	+11	8377 ± 4218 (2003–18,835) *p* = 0.8032	8234 ± 4551 (2298–18,966)	−2	9185 ± 3866 (2405–19,287) *p* = 0.0243	10,415 ± 4758 (2658–23,536)	+13	0.0549	0.0008	0.3278	0.4309
Acrolein	3-HPMA (μg/24 h)	1240 ± 688 (278–3219) *p* = <0.0001	1508 ± 742 (467–3444)	+22	1016 ± 597 (157–2761) *p* = <0.0001	619 ± 279 (48–1188)	−39	1137 ± 651 (285–2854) *p* = <0.0001	596 ± 301 (211–1311)	−48	0.0662	<0.0001	0.3885	<0.0001
1,3-Butadiene	MHBMA (ng/24 h)	3707 ± 2943 (103–16,005) *p* = 0.0106	4336 ± 3556 (311–16,214)	+17	3109 ± 2789 (147–11,375) *p* = <0.0001	1684 ± 1773 (67–7688)	−46	4146 ± 3954 (236–15,869) *p* = <0.0001	1882 ± 1991 (112–8827)	−55	0.3113	<0.0001	0.4532	<0.0001

1,3-Butadiene	DHBMA (μg/24 h)	476 ± 122 (231–728) $p = 0.935$	478 ± 121 (195–743)	+<1	448 ± 140 (209–739) $p = 0.0229$	413 ± 109 (197–689)	−8	463 ± 112 (185–710) $p = 0.0229$	421 ± 111 (184–623)	−9	0.2586	0.0118	0.5971	0.0239
Phenanthrene	2-Hydroxy phenanthrene (ng/24 h)	128 ± 127 (40–723) $p = 0.2393$	109 ± 34 (48–189)	−15	86 ± 36 (41–175) $p = 0.0170$	124 ± 150 (34–996)	+44	100 ± 43 (41–256) $p = 0.4594$	111 ± 46 (47–244	+11	0.0205	0.4112	0.1116	0.9211
Phenanthrene	3-Hydroxy phenanthrene (ng/24 h)	176 ± 78 (56–434) $p = 0.6901$	179 ± 63 (73–374)	+2	150 ± 54 (53–289) $p = 0.0029$	172 ± 68 (63–332)	+15	165 ± 72 (56–351) $p = 0.0214$	181 ± 74 (83–337)	+10	0.0684	0.6493	0.4178	0.8968
Phenanthrene	4-Hydroxy phenanthrene (ng/24 h)	59 ± 24 (18–140) $p = 0.4859$	54 ± 18 (25–109)	−8	43 ± 26 (7–124) $p = 0.2493$	51 ± 25 (16–132)	+19	37 ± 21 (9–91) $p = 0.0001$	63 ± 36 (20–167)	+70	0.0463	0.7374	0.0052	0.2131
Phenanthrene	1+9-Hydroxy phenanthrene (ng/24 h)	418 ± 135 (121–746) $p = 0.4161$	450 ± 158 (198–793)	+8	347 ± 161 (149–918) $p = <0.0001$	524 ± 463 (134–2852)	+51	360 ± 152 (120–730) $p = 0.0367$	429 ± 181 (133–911)	+19	0.1928	0.1649	0.2197	0.7623

*TNeq = total nicotine equivalents; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = N-nitrosonornicotine, NAB = N-nitrosoanabasine; NAT = N-nitrosoanatabine; HMPMA = 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA = 3-hydroxypropylmercapturic acid; DHBMA = 1,2-dihydroxybutyl mercapturic acid; MHBMA = monohydroxybutenyl-mercapturic acids.

For group 4 (TSS1) nine BoE values increased and eight decreased from baseline to end of study, despite there being no case where there was an increase in toxicant yield when measuring the smoke chemistry. The volatile species again showed the greatest statistically significant reductions—58%, 40% and 46% for crotonaldehyde, acrolein and 1,3-butadiene (MHBMA) biomarkers, respectively. Significant increases were seen in concentrations of BoE for nicotine, NNK, 4-aminobiphenyl, 3-aminobipheny, l, 2-amino naphthalene and all but one phenanthrene BoE (Table 5). Of the TSNA BoE, NNK (measured as NNAL) increased significantly between baseline and day 41, while absolute levels of NNN, NAB and NAT BoE all reduced. A significant 19% increase in CPD on day 41 compared with baseline (Table 3) may account for some of these increases.

In group 5 (BT1) significant reductions in levels were seen for 11 BoE. In particular, reductions in BoE of between 46–86% were seen for the TSNAs, up to 38% for aromatic amines, 62% for crotonaldehyde, 48% for acrolein and 55% for 1,3-butadiene (MHBMA) (Table 5). NNAL was not reduced to the same extent as the other TSNAs and while reductions in NNN, NAB and NAT were in line with smoke chemistry reductions, NNAL was not. However, NNAL is a metabolite of NNK with a longer half-life, whilst NNN, NAB and NAT are smoke constituents with short half-lives. We found a significant increase in TNeq of 23%, which was in keeping with the smoke nicotine yields of CC1 and BT1 (Table 1). There were also significant increases in 2-hydroxyfluorene, 1-hydroxynaphthalene and three of the hydroxyphenanthrenes.

3.4. Non-smoker Group

The difference in BoE levels between the smoker and non-smoker groups depended on the BoE. Detectable levels of TNeq and TSNA BoEs were found in the non-smoker group, suggesting some previous level of exposure to environmental tobacco smoke. The mean level of TNeq was around 0.3% of that of the smoker group.

Two BoEs were measured for 1,3-butadiene. The mean level of MHBMA for group 1 was 4501 ng/24 h, compared to the corresponding level in non-smoker (group 6) of 117 ng/24 h, whereas the level of DHBMA was 484 μg/24 h in group 1 compared to 332 μg/24 h in group 6.

For groups 2, 4 and 5, in no case did the BoE level at day 41 reach the level found in group 6, though for BoEs where there were substantial reductions in smoke yields, such as crotonaldehyde, BoEs in these groups became much closer to the mean group 6 level.

3.5. Mid-point data

Full BoE data including data for the mid-point of the study (day 28) are available in additional files 1–4.

In general, levels of BoE did not differ significantly between days 28 and 41 but there were some exceptions (see additional Tables 10, 11 and additional Figs. 6, 7). TNeq for the 1 mg RTP groups increased significantly and systematically from day 14 to day 28 to day 41 and a similar pattern was seen for nicotine MLE values. Results suggest that the elimination half-lives of most of BoE were short, i.e. reductions were achieved within the first 2 weeks following the switch. Since these reductions were generally maintained for the remainder of the study (two timepoints), it suggests that subjects complied with the protocol, and that a shorter period of study could have fulfilled the primary objectives for most BoEs.

3.6. Smoke chemistry vs biomarker changes

Included in Tables 7–9 are the percent changes in toxicant yields as measured on smoking machines (Test vs Control product) displayed alongside the percent changes in biomarkers.

Table 6
LS Means, SD and ranges for biomarkers of exposure in non-smokers (group 6).

Biomarker*	Group 6 (NS)
	Day 56
TNeq (mg/24 h)	0.04 ± 0.04 (0.02–0.26)
NNAL(ng/24 h)	22 ± 61 (1–411)
NNN (ng/24 h)	0.9 ± 0.9 (0.3–5.3)
NAB (ng/24 h)	1.5 ± 3.2 (0.4–22.8)
NAT (ng/24 h)	3.6 ± 16.7 (0.3–115.4)
4-Aminobiphenyl (ng/24 h)	2.0 ± 1.0 (0.5–4.9)
3-Aminobiphenyl (ng/24 h)	0.5 ± 0.3 (0.2–2.3)
o-Toluidine (ng/24 h)	53.9 ± 29.6 (19.3–137.2)
HMPMA (μg/24 h)	189 ± 80 (59–419)
2-Amino naphthalene (ng/24 h)	1.6 ± 0.87 (0.4–3.6)
2-Hydroxy fluorene (ng/24 h)	398 ± 303 (79–1773)
1-Hydroxy pyrene (ng/24 h)	105 ± 65 (37–336)
1-Hydroxy naphthalene (ng/24 h)	545 ± 671 (119–3700)
2-Hydroxy naphthalene (ng/24 h)	3726 ± 4728 (590–26,662)
3-HPMA (μg/24 h)	251 ± 125 (54–5730)
MHBMA (ng/24 h)	117 ± 78 (40–447)
DHBMA (mg/24 h)	332 ± 107 (132–602)
2-Hydroxy phenanthrene (ng/24 h)	96 ± (19–658)
3-Hydroxy phenanthrene (ng/24 h)	104 ± 54 (36–262)
4-Hydroxy phenanthrene (ng/24 h)	37.3 ± 37 (6–208)
1 + 9-Hydroxy phenanthrene (ng/24 h)	282 ± 185 (70–1131)

* TNeq = total nicotine equivalents; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine, NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; HMPMA = 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA = 3-hydroxypropylmercapturic acid; DHBMA = 1,2-dihydroxybutyl mercapturic acid; MHBMA = monohydroxybutenyl-mercapturic acids.

3.6.1. 6 mg Groups

For groups 1 and 2, CC6 and TSS6, the percentage changes in vapour phase toxicant yield corresponded reasonably well with the reductions in BoE levels. For example a 55% reduction in smoke yield of acrolein corresponded to a 44% reduction in the BoE for acrolein, 3-HPMA (Table 7). In the case of 1,3-butadiene the reductions for the BoE MHBMA were greater than the smoke yield reductions.

However, for the particulate phase constituents BoE reductions are typically less, often considerably less, than the smoke yield reductions. For example a reduction in smoke yield of 50% for NNN compared to a reduction of 28% in the NNN BoE.

3.6.2. 1 mg Groups

There were reductions in all toxicant yields for the TSS1 RTP vs the CC1 control although the degree of reduction varied by toxicant (Table 8). The BoE data for group 4 (TSS1) showed varied responses including a reasonable number of increases so comparison between changes in yield and BoE were often not in agreement. For example, there was a reduction in NNK yields comparing TSS1 with CC1, but an increase in NNAL for the group switched from CC1 to TSS1.

In group 5 (BT1), there were generally similar reductions in smoke yields and BoE. Some of these reductions were substantial, and included decreases of greater than 80%.

Reductions in smoke yields and biomarkers for three of the four TSNAs were in agreement: Smoke yield reductions for NNN, NAB and NAT were between 85% and 96%, and reductions in BoE for these toxicants ranged between 81% and 87%. However the NNK reductions in smoke yield of 83% did not correspond well with BoE reductions, which on average were 49% (Table 9). Again, this difference is most likely due to the fact that NNAL is a metabolite with a long half-life, while the other TSNAs are un-metabolised with shorter half-lives.

3.6.3. General observations

For all RTPs, changes in the levels of NNAL and naphthalene did not compare well with changes in the corresponding smoke yields. However NNN, NAB, NAT, crotonaldehyde, acrolein showed clear and stable trends, with significant reductions across all RTPs with respect to both smoke yields and BoEs.

3.7. Variation in individual participant changes

Fig. 4 provides a descriptive example of individual subject data for one BoE: NAT, and illustrates the large range of individual responses seen across the study population. It was common within a group to see some subjects increase BoE levels even if the group as a whole showed a significant decrease, as exemplified by TSS1 results in Fig. 4. The BT1 results in this example are a rare case where all individuals reduced their BoE level for NAT. Consideration of the individual participant data from this study will be the subject of a separate manuscript.

3.8. Sensory data

In general, subjects reported equivalence or lower acceptability for RTPs in most of the sensory categories (Fig. 5). On occasions there was substantially lower acceptability, however overall acceptability of the prototypes seemed to improve over the course of the 4 weeks that the participants were smoking them.

4. Discussion

The primary objective of the study was to estimate and compare cigarette smoke exposure in healthy adult smokers using both BoE and filter analysis, and to quantify any changes in these exposure estimates following a switch from commercial-style to novel cigarettes. 92% of enrolled subjects successfully completed the study to protocol, providing BoE and filter analysis results for statistical analysis.

This proof-of-concept clinical study demonstrates that significant reductions in tobacco smoke toxicant yields observed in machine smoking of RTPs can translate to reductions in BoE levels in smokers. However, yield reductions did not always lead to BoE reductions, and varied widely between individuals.

All three RTPs were associated with reductions in yields of multiple smoke toxicants compared to their control cigarettes and, for some of these, reductions were substantial. BoE levels for the volatile smoke toxicants crotonaldehyde, acrolein and 1,3-butadiene (MHBMA) were substantially reduced in smokers of all three RTP cigarettes as predicted by smoke toxicant yields. Levels of three of the four TSNAs (NNN, NAB, NAT) also showed significant reductions, largely in keeping with the smoke toxicant yields. None of the test products, despite having substantially reduced levels in some smoke toxicants, reduced BoE levels to those found in non-smokers, though in some cases reductions led to levels that came close to non-smoker levels. The control cigarettes were associated with urinary BoE levels that were in keeping with published data for European smokers (Lindner et al., 2011). For groups of individuals who switched to the RTPs, toxicant biomarkers were generally correlated with nicotine biomarkers indicating that uptake of toxicant was determined by concentration in smoke and inhaled amount of smoke. This correlation is not shown here but will be explored in a subsequent paper (in preparation) that considers the correlation between toxicant tield as measured under different machine smoking regimes and human exposure as determined using BoE.

Although reductions were seen across all smoke toxicants in RTPs, compared with their controls, the BoE data showed increases in exposure for several of these toxicants. In the case of TSS1, group 4, there was a distinct increase in consumption per day at day 41,

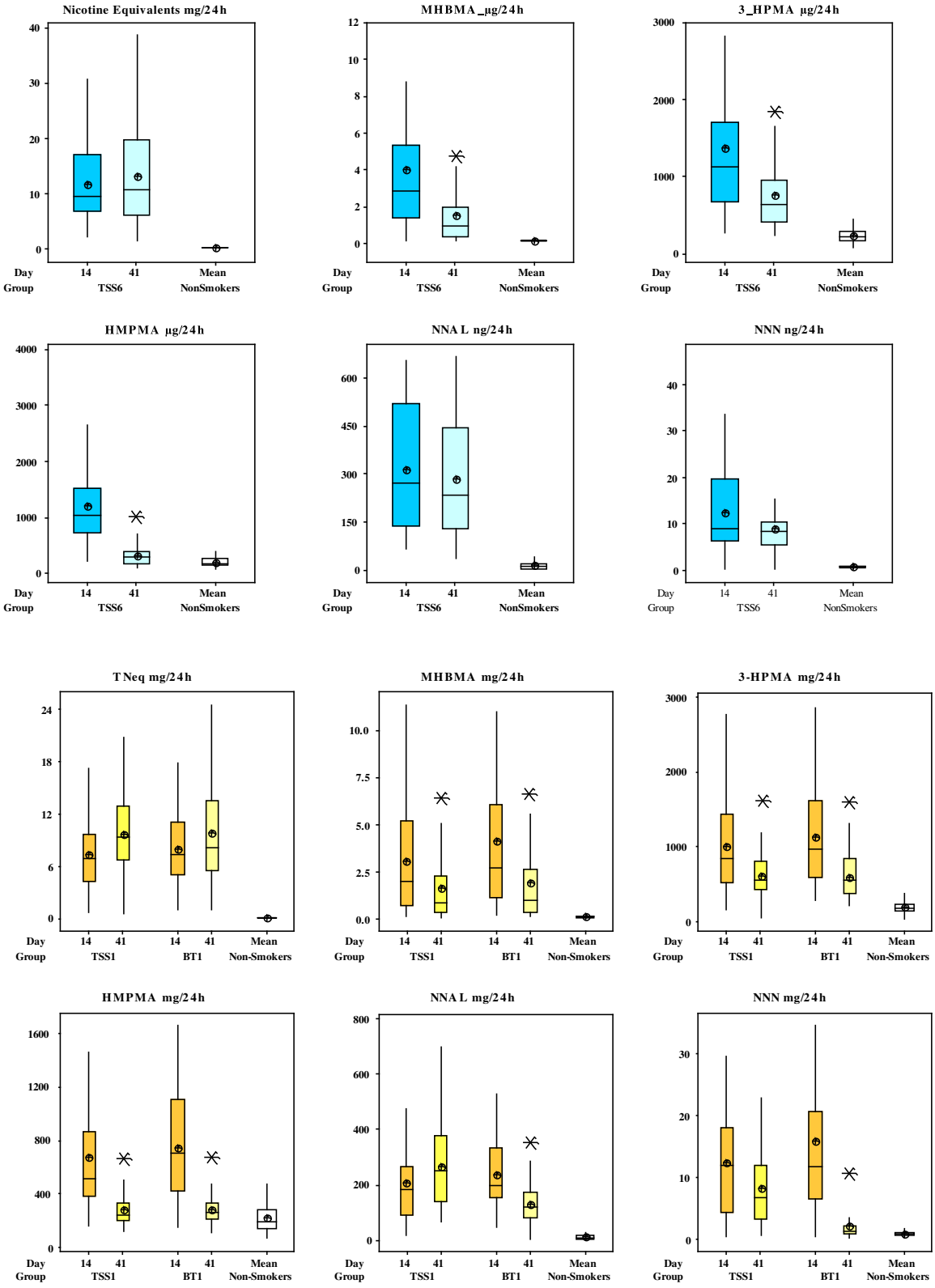

Fig. 3. Boxplots of selected biomarkers. Key: Dark blue – 6mg control cigarette, pale blue – 6mg RTP, Dark Yellow – 1mg control cigarette, pale yellow – 1mg RTP, Unshaded – non-smokers, Line across box = mean. Circle in box = median. Boundaries of box = 25 and 75 percentile. Whiskers = range.* indicates significance of difference from baseline (Day 14), i.e. $p \leqslant 0.05$.

as compared to baseline, and this appeared, as should be expected, to be a key influencer on toxicant exposure.

In this study the BoE and filter analysis samples were acquired during clinical confinement. Clinical confinement may help with compliance to protocol, but smoking in this environment may not reflect natural behaviour even though subjects were free to smoke *ad libitum* in an effort to minimise any potential changes in smoking behaviour. We found increased cigarette consumption at the end of the study, which could be due to a variety of factors including the imminent end of the supply of free cigarettes. Exten-

Table 7
Smoke yields and biomarkers of exposure of 6 mg cigarette groups (CC6 vs TSS6).

Smoke constituent	Smoke yields			Biomarker	Biomarker data		
	CC6	TSS6			Amount: Group 4 (TSS6)		
	Yield	Yield	Δ (%)		Day 14	Day 41	Δ (%)*
Nicotine (mg/cig)	1.6	1.4	−13	Tneq (mg/24 h)	11.7	12.9	10
NNK (ng/cig)	80	44.5	−44	NNAL (ng/24 h)	315	282	−10
NNN (ng/cig)	146.9	72.8	−50	NNN (ng/24 h)	14.8	11.5	−22
NAB (ng/cig)	12.1	7.6	−37	NAB (ng/24 h)	35.3	26.2	−26
NAT (ng/cig)	117.6	69.5	−41	NAT (ng/24 h)	187	143	−24
4-Aminobiphenyl (ng/cig)	3.1	2.7	−13	4-Aminobiphenyl (ng/24 h)	15.7	14.7	−6
3-Aminobiphenyl (ng/cig)	4.1	3.3	−20	3-Aminobiphenyl (ng/24 h)	6.8	6.1	−10
o-Toluidine (ng/cig)	88.1	76.2	−14	*o*-Toluidine (ng/24 h)	143	131	−8
Crotonaldehyde (μg/cig)	45.2	7	−85	HMPMA (μg/24 h)	1215	308	−75
2-Aminonaphthalene (ng/cig)	14.6	14.8	1	2-Amino naphthalene (ng/24 h)	23.7	21.1	−11
Fluorene (ng/cig)	315.7	240.9	−24	2-Hydroxy fluorene (ng/24 h)	2587	2232	−14
Pyrene (ng/cig)	108.1	80.3	−26	1-Hydroxy pyrene (ng/24 h)	283	267	−6
Naphthalene (ng/cig)	2952.3	565.6	−81	1-Hydroxy naphthalene (ng/24 h)	7103	6065	−15
				2-Hydroxy naphthalene (ng/24 h)	13904	12323	−11
Acrolein (mg/cig)	139.4	62.3	−55	3-HPMA (μg/24 h)	1365	751	−45
				MHBMA (ng/24 h	4028	1487	−63
1,3-Butadiene (mg/cig)	63.6	36.8	−42	DHBMA mg/24 h)	440	407	−8
				2-Hydroxy phenanthrene (ng/24 h)	113	108	−4
Phenanthrene (ng/cig)	739.8	589.7	−20	3-Hydroxy phenanthrene (ng/24 h)	217	207	−5
				4-Hydroxy phenanthrene (ng/24 h)	55	68	24
				1+9-hydroxy phenanthrene (ng/24 h)	578	526	−9

* Δ (%) data are the means of the per subject percentage changes.

Table 8
Smoke yields and biomarkers of exposure of 1 mg cigarette groups, part 1 (CC1 vs TSS1).

Smoke constituent	Smoke yields			Biomarker	Biomarker data		
	CC1	TSS1			Amount: Group 4 (TSS1)		
	Yield	Yield	Δ (%)		Day 14	Day 41	Δ (%)*
Nicotine (mg/cig)	1.3	1.2	−8	Tneq (mg/24 h)	7.3	9.8	34
NNK (ng/cig)	57.9	48.2	−17	NNAL (ng/24 h)	207	271	31
NNN (ng/cig)	245.2	76	−69	NNN (ng/24 h)	12.2	8.3	−32
NAB (ng/cig)	13.6	6.6	−51	NAB (ng/24 h)	23.5	20.5	−13
NAT (ng/cig)	124.5	70.3	−44	NAT (ng/24 h)	144	99.0	−31
4-Aminobiphenyl (ng/cig)	2.8	2.5	−11	4-Aminobiphenyl (ng/24 h)	10.1	12.6	25
3-Aminobiphenyl (ng/cig)	3.5	3	−14	3-Aminobiphenyl (ng/24 h)	4.2	5.4	29
o-Toluidine (ng/cig)	68.1	60.1	−12	*o*-Toluidine (ng/24 h)	122	111	−9
Crotonaldehyde	41.6	6	−86	HMPMA (μg/24 h)	680	288	−58
2-Aminonaphthalene (ng/cig)	13.1	11.5	−12	2-Amino naphthalene (ng/24 h)	15.0	19.6	30
Fluorene (ng/cig)	230.5	148.3	−36	2-Hydroxy fluorene (ng/24 h)	1627	1751	8
Pyrene (ng/cig)	70.4	64.6	−8	1-Hydroxy pyrene (ng/24 h)	175	190	9
Naphthalene (ng/cig)	2182.5	643.8	−71	1-Hydroxy naphthalene (ng/24 h)	4201	4213	<1
				2-Hydroxy naphthalene (ng/24 h)	8377	8234	−2
Acrolein (μg/cig)	130.5	52.5	−60	3-HPMA (μg/24 h)	1016	619	−39
1,3-Butadiene (μg/cig)	39.6	27.2	−31	MHBMA (ng/24 h)	3109	1684	−46
				DHBMA (μg/24 h)	448	413	−8
Phenanthrene (ng/cig)	524.4	191.4	−64	2-Hydroxy phenanthrene (ng/24 h)	86	124	44
				3-Hydroxy phenanthrene (ng/24 h)	150	172	15
				4-Hydroxy phenanthrene (ng/24 h)	43	51	19
				1 + 9-Hydroxy phenanthrene (ng/24 h)	347	524	51

* Δ (%) data are the means of the per subject percentage changes.

sion of study duration, to incorporate days that are not used for data analysis, might be useful to avoid increased consumption affecting results.

BoE data will not only reflect behaviour during confinement but, depending on the half-life of the biomarker, may also reflect behaviour in the days immediately before clinical confinement. Most of the biomarkers have a short half-life however NNK is an exception. It has been reported as biphasic with a first-phase elimination ($t_{1/2}\alpha$) occurring between 14 h and 3.3–4.0 days and second-phase elimination ($t_{1/2}\beta$) between 10–18 days and 39–45 days (Goniewicz et al., 2009; Hecht et al., 1999). This may explain the discrepancies in our data for NNK. Owing to the timescale of this study, measurement of NNAL is unlikely to have fully reflected the exposure to NNK after switching. The behaviours of NNAL and the two BoE for naphthalene were the least consistent with smoke yield changes. Differences in the NNK smoke yields consistently overestimated the total reductions in NNAL achievable with RTPs, by around 50%, although for TSS1 the reduction in NNK yield was associated with an increase in NNAL challenging the notion that this is simply a half-life effect. The finding was pronounced with the two naphthalene biomarkers, where naphthalene smoke yields were 70–80% lower with RTPs than with control cigarettes, but under real-life conditions the differences for 1-hydroxynaphthalene and 2-hydroxynaphthalene were less than 30%. A similar explanation is unlikely for 1-hydroxynaphthalene

Table 9
Smoke yields and biomarkers of exposure of 1 mg cigarette groups, part 2 (CC1 vs BT1).

Smoke constituent	Smoke yields			Biomarker	Biomarker data		
	CC1	BT1			Amount: Group 5 (BT1)		
	Yield	Yield	Δ (%)		Day 14	Day 41	Δ (%)*
Nicotine (mg/cig)	1.3	1.5	15	Tneq (mg/24 h)	8.0	9.8	23
NNK (ng/cig)	57.9	10.1	−83	NNAL (ng/24 h)	238	128	−46
NNN (ng/cig)	245.2	10.2	−96	NNN (ng/24 h)	15.8	2.2	−86
NAB (ng/cig)	13.6	1.4	−90	NAB (ng/24 h)	30.1	5.9	−80
NAT (ng/cig)	124.5	19.1	−85	NAT (ng/24 h)	186	28.5	−85
4-Aminobiphenyl (ng/cig)	2.8	1.2	−57	4-Aaminobiphenyl (ng/24 h)	11.1	6.9	−38
3-Aminobiphenyl (ng/cig)	3.5	1.8	−49	3-Aminobiphenyl (ng/24 h)	4.3	2.7	−37
o-Toluidine (ng/cig)	68.1	50.6	−26	*o*-Toluidine (ng/24 h)	130	124	−5
Crotonaldehyde	41.6	10.9	−74	HMPMA (μg/24 h)	741	275	−62
2-Aminonaphthalene (ng/cig)	13.1	7.4	−44	2-Amino naphthalene (ng/24 h)	16.3	13.8	−15
Fluorene (ng/cig)	230.5	247.3	7	2-Hydroxy fluorene (ng/24 h)	1794	2134	19
Pyrene (ng/cig)	70.4	75.3	7	1-Hydroxy pyrene (ng/24 h)	190	197	4
Naphthalene (ng/cig)	2182.5	484.9	−78	1-Hydroxy naphthalene (ng/24 h)	4847	5744	16
				2-hydroxy naphthalene (ng/24 h)	9184	10,415	13
Acrolein (μg/cig)	130.5	75	−43	3-HPMA (μg/24 h)	1137	596	−48
1,3-Butadiene (μg/cig)	39.6	53.4	35	MHBMA (ng/24 h)	4146	1882	−55
				DHBMA (μg/24 h)	463	421	−9
Phenanthrene (ng/cig)	524.4	541.5	3	2-Hydroxy phenanthrene (ng/24 h)	100	111	11
				3-Hydroxy phenanthrene (ng/24 h)	165	181	10
				4-Hydroxy phenanthrene (ng/24 h)	37	63	70
				1 + 9-Hydroxy phenanthrene (ng/24 h)	360	429	19

* Δ (%) data are the means of the per subject percentage changes.

and 2-hydroxynaphthalene, where an elimination half-life of 25.7 h has been reported in workers exposed to hot asphalt (Sobus et al., 2009).

Levels of DHBMA and all BoE for phenanthrene were similar in the smoker and non-smoker groups. This observation has been reported previously (U.S. Department of Health and Human Services, 2010). The high levels in non-smokers were presumably related to environmental and/or dietary sources of exposure, and may limit the resolving power of these biomarkers as measures of smoke toxicant exposure reduction. As none of the biomarkers show good selectivity for phenanthrene, it is questionable whether any should be included in future studies of this kind. For 1,3-butadiene, MHBMA appeared to have much greater selectively than DHBMA and, in keeping with previously published opinion (U.S. Department of Health and Human Services, 2010) it must be concluded that DHBMA is not a viable biomarker for 1,3-butadiene.

The filter analysis MLE data for the 6 mg control cigarettes and RTPs were in line with the smoke yields and TNeqs whilst, in contrast the 1 mg controls and RTPs were not. In principle the filter analysis methodology should not be influenced by the filter constructions of the 1 mg RTPs. If smokers of the 1 mg RTP puffed at a flow rate substantially different to that used during method calibration, then estimates of MLE can be less accurate. However, limited puffing topography data collected during this study (data not show) indicated that smokers smoked the RTP cigarettes in manner that was broadly similar to the control cigarettes. Even so, application of the filter analysis method can become more challenging with highly-ventilated 1 mg ISO tar yield products, and the applicability of this assay to very novel filter designs warrants further study.

Despite the general increase in nicotine measures for all the 1 mg groups, the per cigarette data (0.63–0.89 mg/cig nicotine MLE and 0.43–0.52 mg/cig TNeq (per cig data not shown)) remained in keeping with previously published MLE and TNeq data for a commercial 1 mg cigarette in Germany (0.82 and 0.40 mg/cig, respectively Shepperd et al., 2009).

Variation between individuals in this study was large and presumably reflects both individual smoking behaviour and inter-individual variation in metabolism. These variations mean that while group mean BoE may reduce, not all subjects in the group actually experiences decreases in BoE levels. There were no health end-points in this study, therefore the likely impact on long term health risks which might be associated with reducing some but not all tobacco smoke toxicants cannot be assessed. Longer term studies of RTPs that include markers of biological relevance should be considered.

The US Food and Drug Administration (FDA) have been developing guidelines for applications related to modified risk tobacco products (MRTPs) either related to reduced exposure to toxicants or reduced health risks. As part of the development of these guidelines, the FDA commissioned the Institute of Medicine to produce a report on scientific standards in studies of MRTPs, informed by a series of studies and reviews by Hatsukami and others, that set out the methodological challenges of assessing MRTPs (Hatsukami et al., 2006, 2009). The FDA has recently provided draft guidance on both what are harmful and potentially harmful constituents in tobacco and tobacco smoke (FDA Guidance for Industry, 2012a), and on what studies are needed to assess modified risk tobacco products (FDA Guidance for Industry, 2012b). The measurement of BoE of tobacco smoke toxicants could be important to regulatory approaches related both to the assessment of potential MRTPs and to approaches seeking to reduce exposures to tobacco smoke toxicants through the setting of product standards.

This study, although demonstrating reductions in the BoE for a number of smoke toxicants is clearly insufficient to meet the level of scientific evidence required to allow the products to be assessed as an MRTP, under IoM, FDA and our own proposed framework of scientific assessment for such products (Proctor and Ward, 2011). It does illustrate that more work is still needed on the methodology employed in clinical studies designed to assess tobacco products with reduced toxicant levels. Additional BoEs should be developed to represent important toxicants, and the performance of some existing BoEs could be improved by measuring additional metabolites, or at least developing a greater understanding of metabolic pathways for some toxicants. Since the suite of nicotine plus five metabolites are known to amount to approximately 80% of nicotine intake (Benowitz et al., 1994) it is conventional to sum as TNeq (Total nicotine

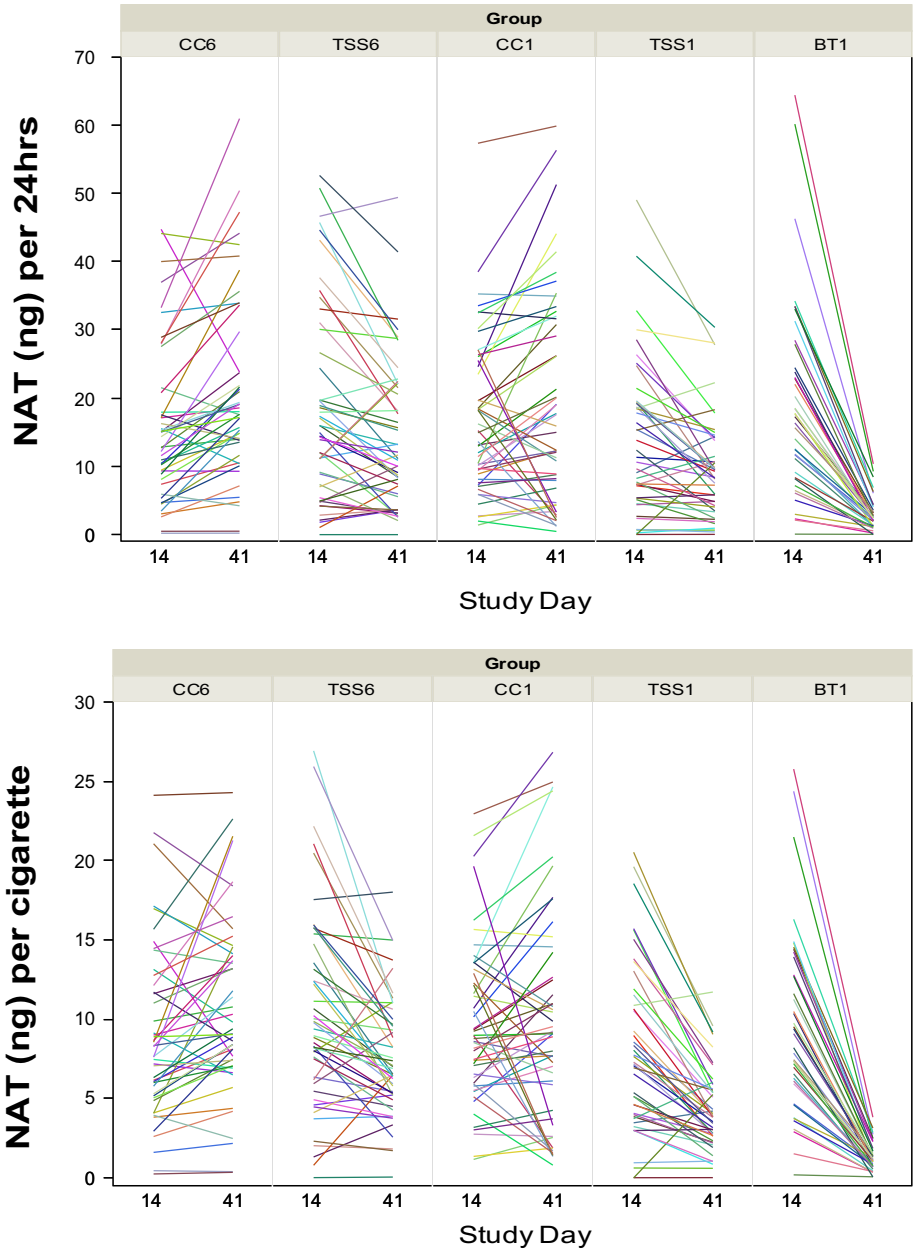

Fig. 4. Individual subject results for NAT BoE per day (top plot) and per cigarette (bottom plot).

equivalents). Improved mass balance with additional metabolites would therefore be an advantage.

A study design that includes occasional clinical confinement seems to influence daily cigarette consumption, especially as the study comes to a close, and daily consumption can have an important impact on BoE levels, suggesting designs that run longer than needed for the clinical assessment might be necessary.

Most importantly, although this study shows that reductions in smokers' exposure to toxicants as measured by BoE is possible by reducing smoke toxicant yields, more research is needed to assess whether such reductions will have an effect on health risks. Such research should, as a first step, include biomarkers of biological effect, that being endogenous compounds released by the body in response to stress such as toxicant exposure. These might include F2-isoprostane (for oxidative stress) and sICAM-1 and white blood cell count for cardiovascular disease.

5. Conclusions

The primary objective of the study was to estimate and compare cigarette smoke exposure in healthy adult smokers by assessment of BoE and mouth level exposure (MLE) to tar and nicotine (as measured by filter analysis), and to quantify any changes in exposure after switching from control cigarettes to RTPs.

The study found that large reductions in smoke toxicant yields can reduce mean BoE levels in groups of smokers (as seen in the BoE levels of vapour phase toxicants), but that smoking behaviour remains an important determinant of toxicant exposure and that cigarettes that demonstrate reductions in toxicant yields as measured by smoking machines can still result in increases in BoE levels in groups of smokers. Generally, where the reductions in toxicant levels were very large, as was the case for acrolein and crotonaldehyde, there were corresponding large reductions in

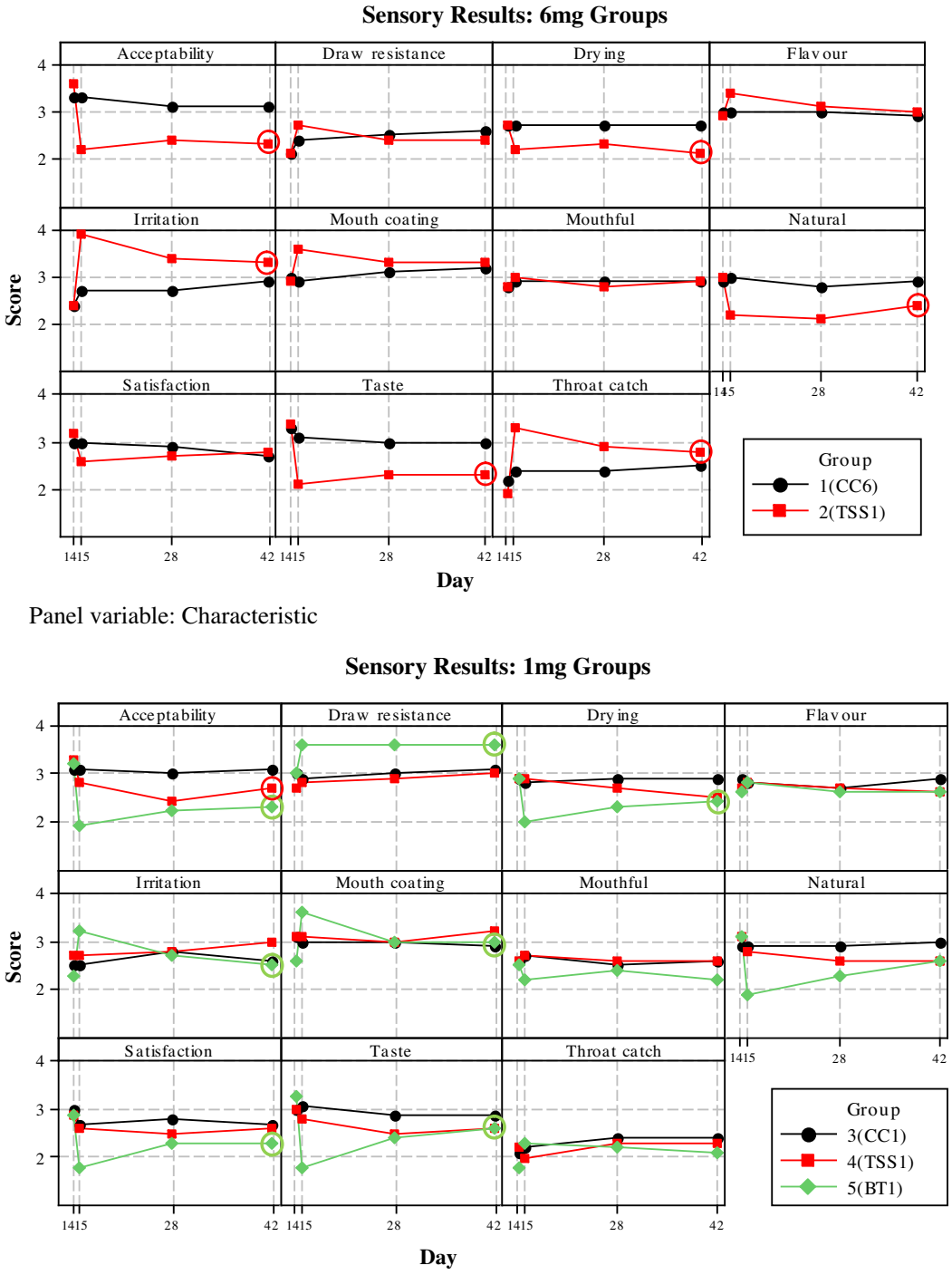

Fig. 5. Mean sensory questionnaire scores for all products by study group. Indicates a significant difference between Day 14 and Day 42 sensory scores.

BoE levels. Where reductions in toxicants levels were modest, as was more likely to be seen for many of the particulate phase toxicants, mean BoE may still increase following a switch from a conventional cigarette to a RTP. Increases in daily cigarette consumption or other more-intensive smoking behaviours can undermine the reductions seen in toxicant yields. This study confirms that individual smoking behaviours vary widely, and this, perhaps in addition to other factors such as variations in metabolism, results in wide ranges in BoE levels across the smoker population.

Not all BoEs behaved the same. This was particularly true across the range of tobacco-specific nitrosamine BoEs, where NNAL gave quite different results from the other TSNA BoEs. This suggests that in such studies a range of biomarkers should be measured.

MLE levels corresponded with BoE measures of nicotine exposure for the higher ISO tar yield products (RTP and control) but not for the lower ISO tar yield RTPs, and more research is needed into the methodology used in MLE for very novel products.

The reductions in BoE for smoke toxicants demonstrated in this study show that it is possible to reduce exposure to some tobacco

smoke toxicants, as measured by smoke chemistry, by switching smokers from conventional cigarettes to RTPs. However, the study found considerable variability in both absolute BoE levels and percentage reductions, in some cases BoE increased and in no case were the reductions in toxicant exposure so large as to reduce exposures to non-smoker levels. Further evaluation should be undertaken on the impact of cigarettes with substantially reduced levels of tobacco smoke toxicants on longer-term behaviours, and such studies should include end points that assess the possible effects of reductions on health.

Conflict of interest statement

CJS, AE, OMC, KMcA and CJP are current employees of British American Tobacco, and the work was funded by British American Tobacco. IM was the principal investigator at the clinic where the study was carried out and is employed by Momentum Pharma Services in Hamburg, Germany.

Author contributions

CJS and AE contributed substantially to the study design, project managed the study and, along with the other authors, co-wrote the manuscript. OMC and KMcA contributed to the data interpretation and co-wrote the manuscript. CJP directed the research and co-wrote the manuscript, and IM was the principal investigator at the clinical site.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2013.02.007.

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